WEST Search History

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DATE: Monday, July 19, 2004

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,EPAB,JPAB,DWPI; PLUR=YES; OP=OR	
☐	L9	L8 not (l2 or l3)	27
☐	L8	L6 and (metalloprot$9 or autocleav$6 or NS2 with NS3 or NS2/3 or NS2/ns3 or p21 with p70) same (inhibit$6 or antagon$8)	73
☐	L7	L6 and (metalloprot$9 or autocleav$6 or NS2 with NS3 or NS2/3 or NS2/ns3 or p21 with p70)	137
☐	L6	(protease or proteinase or autoclaev$6) with HCV with (inhibit$6 or antagon$8)	295
☐	L4	l2 not l3	32
☐	L3	((soybean adj trypsin adj inhibitor or EDTA or TPCK) same (Cpro-1 or HCV with (protease or proteinase or autocleavage or metalloprot$9 or NS2/3 or NS2/ns3)))	40
☐	L2	(inhibitor same (Cpro-1 or HCV with (autocleavage or metalloprot$9 or NS2/3 or NS2/ns3)))	50
☐	L1	((soybean adj trypsin adj inhibitor or EDTA or TPCK) same (Cpro-1 or HCV with (protease or proteinase or autocleavage or metalloprot$9 or NS2/2 or NS2/ns3)))	40

END OF SEARCH HISTORY

STN Search History

FILE 'HOME' ENTERED AT 12:03:17 ON 19 JUL 2004

L1 3159 (HCV OR HEPATITIS (N) (C OR NON-A (A) NON-B OR NANB)) (P) (PROTE ASE OR POLYPROTEIN (S) PROCESS! OR PROTEINASE OR CPRO-1 OR METAL LOPROT! OR NS2/3 OR NS2/NS3 OR P21/P70)

L2 3159 (HCV OR HEPATITIS (N) (C OR NON-A (A) NON-B OR NANB)) (P) (PROTE ASE OR POLYPROTEIN (S) PROCESS! OR PROTEINASE OR CPRO-1 OR METAL LOPROT!)

L3 1848 L1 AND (PROTEASE OR PROTEINASE OR PROCESS!) (S) (INHIBIT#### OR ANTAGON#####)

L4 330 L2 AND (AUTOCLEAV#### OR METALLOPROT########## OR NS2 OR P21 OR (NONSTRUCTURAL OR NON-STRUCTURAL) (A) PROTEIN (A) 2)

L9 29 L1 AND (METALLOPROT####### OR AUTOCLEAV#######) (S) (INHIBIT#### # OR BLOCK##### OR ANTAGON##### OR PREVENT)

L12 32 L3 AND (METALLOPROT####### OR AUTOCLEAV#######)

(FILE 'HOME' ENTERED AT 12:03:17 ON 19 JUL 2004)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 12:03:52 ON 19 JUL 2004

L1 3159 S (HCV OR HEPATITIS (N) (C OR NON-A (A) NON-B OR NANB)) (P) (PR
L2 3159 S (HCV OR HEPATITIS (N) (C OR NON-A (A) NON-B OR NANB)) (P) (PR
L3 1848 S L1 AND (PROTEASE OR PROTEINASE OR PROCESS!) (S) (INHIBIT####
L4 330 S L2 AND (AUTOCLEAV#### OR METALLOPROT########## OR NS2 OR P21
L5 130 S L4 AND ("NS2/3" OR NS2/NS3)
L6 216 S L4 AND ("NS2/3" OR "NS2/NS3")
L7 77 DUP REM L6 (139 DUPLICATES REMOVED)
L8 45 S L7 NOT PY>1999
L9 29 S L1 AND (METALLOPROT####### OR AUTOCLEAV#######) (S) (INHIBIT#
L10 28 S L9 NOT L8
L11 18 DUP REM L10 (10 DUPLICATES REMOVED)
L12 32 S L3 AND (METALLOPROT####### OR AUTOCLEAV#######)
L13 6 S L12 NOT (L9 OR L8)
L14 0 S L13 NOT PY>1999

L8 ANSWER 4 OF 45 MEDLINE on STN
AN 2000044691 MEDLINE
DN PubMed ID: 10574908
TI Inhibition of hepatitis C virus **NS2/3** processing by NS4A peptides. Implications for control of viral processing.
AU Darke P L; Jacobs A R; Waxman L; Kuo L C
CS Department of Antiviral Research, Merck Research Laboratories, West Point, Pennsylvania 19486, USA.
SO Journal of biological chemistry, (1999 Dec 3) 274 (49) 34511-4. Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200002
ED Entered STN: 20000209
Last Updated on STN: 20000209
Entered Medline: 20000203
AB The **NS2/3 protease** of **hepatitis C** virus is responsible for a single cleavage in the viral polyprotein between the nonstructural proteins **NS2** and NS3. The minimal protein region necessary to catalyze this cleavage includes most of **NS2** and the N-terminal one-third of NS3. **Autocleavage** reactions using **NS2/3** protein translated in vitro are used here to investigate the inhibitory potential of peptides likely to affect the reaction. Peptides representing the cleaved sequence have no effect upon reaction rates, and the reaction rate is insensitive to dilution. Both results are consistent with prior suggestions that the **NS2/3** cleavage is an intramolecular reaction. Surprisingly, peptides containing the 12-amino acid region of NS4A responsible for binding to NS3 inhibit the **NS2/3** reaction with K(i) values as low as 3 microM. Unrelated peptide sequences of similar composition are not inhibitory, and neither are peptides containing incomplete segments of the NS4A region that binds to NS3. Inhibition of **NS2/3** by NS4A peptides can be rationalized from the organizing effect of NS4A on the N terminus of NS3 (the **NS2/3** cleavage point) as suggested by the known three-dimensional structure of the NS3 **protease** domain (Yan, Y., Li, Y., Munshi, S., Sardana, V., Cole, J. L., Sardana, M., Steinkuhler, C., Tomei, L., De Francesco, R., Kuo, L. C., and Chen, Z. (1998) Protein Sci. 7, 837-847). These findings may imply a sequential order to proteolytic maturation events in **hepatitis C** virus.

L8 ANSWER 5 OF 45 MEDLINE on STN
AN 2000039338 MEDLINE
DN PubMed ID: 10574181
TI The design and synthesis of potent inhibitors of **hepatitis C** virus NS3-4A **proteinase**.
AU Attwood M R; Bennett J M; Campbell A D; Canning G G; Carr M G; Conway E; Dunsdon R M; Greening J R; Jones P S; Kay P B; Handa B K; Hurst D N; Jennings N S; Jordan S; Keech E; O'Brien M A; Overton H A; King-Underwood J; Raynham T M; Stenson K P; Wilkinson C S; Wilkinson T C; Wilson F X
CS Department of Chemistry, Roche Discovery Welwyn, Welwyn Garden City, Hertfordshire, UK.
SO Antiviral chemistry & chemotherapy, (1999 Sep) 10 (5) 259-73. Journal code: 9009212. ISSN: 0956-3202.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals

EM 199912
ED Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991209
AB **Hepatitis C** virus (**HCV**) is the cause of the majority of transfusion-associated hepatitis and a significant proportion of community-acquired hepatitis worldwide. Infection by **HCV** frequently leads to persistent infections that result in a range of clinical conditions including an asymptomatic carrier state, severe chronic active hepatitis, cirrhosis and, in some cases, hepatocellular carcinoma. The **HCV** genome consists of a single-stranded, positive sense RNA containing an open reading frame of approximately 9060 nucleotides. This is translated into a single polyprotein of approximately 3020 amino acids (C-E1-E2-p7-**NS2-NS3** -NS4A-NS4B-NS5A-NS5B), which in turn is processed by a series of host and viral **proteinases** into at least 10 cleavage products. The N-terminal portion of the NS3 protein encodes a serine **proteinase** that is responsible for the cleavage at the NS3-4A, NS4A-4B, NS4B-5A and NS5A-5B junctions. The 54 amino acid NS4A protein is a cofactor that binds to the NS3 protein and enhances its proteolytic activity. This report describes the expression of a recombinant NS3-4A **proteinase** fusion protein in Escherichia coli and the in vitro characterization of the enzyme activity using synthetic peptide substrates. It then demonstrates how these results were employed to guide the design of potent inhibitors of this enzyme.

L8 ANSWER 7 OF 45 MEDLINE on STN
AN 1999143345 MEDLINE
DN PubMed ID: 9986797
TI Internal processing of hepatitis C virus NS3 protein.
AU Shoji I; Suzuki T; Sato M; Aizaki H; Chiba T; Matsuura Y; Miyamura T
CS Department of Virology II, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo, 162-8640, Japan.
SO Virology, (1999 Feb 15) 254 (2) 315-23.
Journal code: 0110674. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199903
ED Entered STN: 19990324
Last Updated on STN: 20000303
Entered Medline: 19990309
AB **Hepatitis C** virus (**HCV**) NS3 protein contains at least three enzymatic activities: **NS2-3** **protease**, NS3 serine **protease**, and NTPase/RNA helicase. It has been shown that **NS2/3** cleavage is mediated by **NS2-3 protease**, whereas NS3 serine **protease** is responsible for the other four cleavage sites of the nonstructural (NS) region. In this study, we showed that the internal cleavage of NS3 protein produced two products of 49 kDa (NS3a) and 23 kDa (NS3b) when the entire NS3 region (aa 1027-1657) or the whole open reading frame (aa 1-3010) was expressed in mammalian and insect cells. By means of site-directed mutagenesis, we demonstrated that NS3a/NS3b cleavage occurs within the RNA helicase sequence motif that is highly conserved in the Flaviviridae family and that neither **NS2-3** **protease** nor NS3 serine **protease** was responsible for this cleavage. The NS3 **protease** of flaviviruses, dengue virus type 2, for example, has been shown to mediate the internal cleavage of NS3. The NS3 proteins of **HCV** and dengue virus may thus be cleaved internally at the same sequence by different mechanisms of

proteolysis. Also discussed is a possible role for the internal processing of **HCV** NS3 in the viral life cycle and its pathogenesis.

L8 ANSWER 10 OF 45 MEDLINE on STN
AN 1998111692 MEDLINE
DN PubMed ID: 9450039
TI **Hepatitis C** virus **NS2-3 proteinase**.
AU Wilkinson C S
CS Roche Discovery Welwyn, Welwyn Garden City, Herts, England.
SO Biochemical Society transactions, (1997 Nov) 25 (4) S611.
Journal code: 7506897. ISSN: 0300-5127.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199804
ED Entered STN: 19980422
Last Updated on STN: 20000303
Entered Medline: 19980413

L8 ANSWER 12 OF 45 MEDLINE on STN
AN 97404642 MEDLINE
DN PubMed ID: 9261354
TI In vitro study of the **NS2-3 protease** of **hepatitis C** virus.
AU Pieroni L; Santolini E; Fipaldini C; Pacini L; Migliaccio G; La Monica N
CS I.R.B.M. Instituto di Ricerche di Biologia Molecolare P. Angeletti, Italy.
SO Journal of virology, (1997 Sep) 71 (9) 6373-80.
Journal code: 0113724. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199709
ED Entered STN: 19970926
Last Updated on STN: 20000303
Entered Medline: 19970917
AB Processing at the C terminus of the **NS2** protein of **hepatitis C** virus (**HCV**) is mediated by a virus-encoded **protease** which spans most of the **NS2** protein and part of the NS3 polypeptide. In vitro cotranslational cleavage at the 2-3 junction is stimulated by the presence of microsomal membranes and ultimately results in the membrane insertion of the **NS2** polypeptide. To characterize the biochemical properties of this viral **protease**, we have established an in vitro assay whereby the **NS2-3 protease** of **HCV** BK can be activated posttranslationally by the addition of detergents. The cleavage proficiency of several deletion and single point mutants was the same as that observed with microsomal membranes, indicating that the overall sequence requirements for proper cleavage at this site are maintained even under these artificial conditions. The processing efficiency of the **NS2-3 protease** varied according to the type of detergent used and its concentration. Also, the incubation temperature affected the cleavage at the 2-3 junction. The autoproteolytic activity of the **NS2-3 protease** could be inhibited by alkylating agents such as iodoacetamide and

N-ethylmaleimide. Metal chelators such as EDTA and phenanthroline also inhibited the viral enzyme. The EDTA inhibition of **NS2-3** cleavage could be reversed, at least in part, by the addition of ZnCl2 and CdCl2. Among the common **protease** inhibitors tested, tosyl phenylalanyl chloromethyl ketone and soybean trypsin inhibitor inactivated the **NS2-3 protease**. By means of gel filtration analysis, it was observed that the redox state of the reaction mixture greatly influenced the processing efficiency at the 2-3 site and that factors present in the rabbit reticulocyte lysate, wheat germ extract, and HeLa cell extract were required for efficient processing at this site. Thus, the in vitro assay should allow further characterization of the biochemical properties of the **NS2-3 protease** of **HCV** and the identification of host components that contribute to the efficient processing at the 2-3 junction.

L8 ANSWER 17 OF 45 MEDLINE on STN
AN 96266415 MEDLINE
DN PubMed ID: 8661414
TI In vitro studies on the activation of the **hepatitis C** virus NS3 **proteinase** by the NS4A cofactor.
AU Koch J O; Lohmann V; Herian U; Bartenschlager R
CS Institute for Virology, Johannes-Gutenberg University Mainz, Germany.
SO Virology, (1996 Jul 1) 221 (1) 54-66.
Journal code: 0110674. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199608
ED Entered STN: 19960822
Last Updated on STN: 19960822
Entered Medline: 19960814
AB Proteolytic processing of the nonstructural proteins of the **hepatitis C** virus (**HCV**) is mediated by two viral **proteinases**: the **NS2-3** **proteinase** cleaving at the **NS2/3** junction and the NS3 serine-type **proteinase** responsible for processing at the NS3/4A, NS4A/B, NS4B/5A, and NS5A/B sites. Activity of the NS3 **proteinase** is modulated by NS4A. In the absence of this cofactor processing at the NS3-dependent sites does not occur or, in the case of the NS5A/B junction, is poor but increased when NS4A is present. Although recent studies demonstrated that **proteinase** activation requires direct interaction between NS3 and NS4A, the mechanism by which NS4A exerts the activation function is not known. To further analyze the conditions of **proteinase** activation and to characterize the NS3 sequences important for complex formation and activation we used an in vitro assay in which radiolabeled **HCV** substrates were mixed with NS3 **proteinase** and synthetic NS4A peptides. We found that microsomal membranes are not required for **proteinase** activation. However, they are important for efficient accessibility of the NS4A/B site but not the other trans-cleavage sites. Studies with NS3 deletion mutants identified a region between amino acids 15 and 22 which is essential for **proteinase** activation. Results obtained with several mutations introduced into this sequence show that a weak overall association between NS3 and NS4A is sufficient for **proteinase** activation and suggest that a beta-sheet at the NS3 amino terminus plays an important role. Although not essential for **proteinase** activation the amino terminal 14 NS3 residues were found to have an auxiliary function probably by stabilizing the NS3/4A interaction. Finally, we could demonstrate

intracellular, peptide-mediated modulation of **proteinase** activity providing the basis for the development of a novel therapeutic concept.

L8 ANSWER 21 OF 45 MEDLINE on STN
AN 95088591 MEDLINE
DN PubMed ID: 7996139
TI Analysis of NS3-mediated processing of the hepatitis C virus non-structural region in vitro.
AU D'Souza E D; O'Sullivan E; Amphlett E M; Rowlands D J; Sangar D V; Clarke B E
CS Department of Molecular Sciences, Wellcome Research Laboratories, Beckenham, Kent, U.K.
SO Journal of general virology, (1994 Dec) 75 (Pt 12) 3469-76.
Journal code: 0077340. ISSN: 0022-1317.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199501
ED Entered STN: 19950126
Last Updated on STN: 20000303
Entered Medline: 19950113
AB The **protease** activity of the **hepatitis C** virus (**HCV**) NS3 protein has been investigated using transient expression methods in mammalian cells, as well as in vitro transcription/translation systems. We confirmed that expression of the NS3-5 polyprotein in rabbit reticulocyte lysates results in efficient cis processing at the NS3/NS4 junction. However, processing at the other predicted sites of NS3-mediated cleavage varied markedly in efficiency, the site most susceptible being that between NS5A and NS5B. Time-course analysis of the proteolytic processing of the **HCV** non-structural precursor showed that the cis cleavage between NS3 and NS4 occurred extremely rapidly. However, efficient cleavage at this position was dependent on the prior removal of the **NS2** protein. Furthermore, the presence of uncleaved **NS2** sequences on the enzyme severely impeded NS3-mediated proteolysis at downstream sites in the polyprotein. This suggests therefore that efficient cleavage at the **NS2/NS3** junction is a pivotal event in **HCV** replication. During the course of this study a proteolytically inactive mutant of NS3 was characterized carrying a previously unreported amino acid substitution near the proposed active site of the enzyme. Molecular modelling suggested that the amino acid present at this position may influence the conformation of the active site of the enzyme. Recently a number of reports have described a second **protease** activity, located in the **NS2/NS3** region, which is responsible for cleavage at the **NS2/NS3** junction. We have identified an isolate of **HCV**, obtained from a U.K. patient, which has a virtually inactive **NS2/NS3 protease**. The possible implications of this observation are discussed.

L8 ANSWER 28 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:90406 CAPLUS
DN 132:290272
TI Expression and characterization of the **HCV NS2 protease**
AU Reed, Karen E.; Rice, Charles M.
CS Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA
SO Methods in Molecular Medicine (1998), 19(Hepatitis C Protocols), 331-342

CODEN: MMMEFN
PB Humana Press Inc.
DT Journal
LA English
AB This article describes the use of cell-free transcription and translation (rabbit reticulocyte lysate) systems for the expression and characterization of the **hepatitis C** virus **NS2 protease**.
RE.CNT 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 29 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:604367 CAPLUS
DN 132:46612
TI **Proteases** of the **hepatitis C** virus
AU Urbani, Andrea; De Francesco, Raffaele; Steinkuhler, Christian
CS Inst. di Ricerche di Biologia Molecolare (IRBM) P. Angeletti, Rome, Italy
SO Proteases of Infectious Agents (1999), 61-91. Editor(s): Dunn, Ben M. Publisher: Academic, San Diego, Calif.
CODEN: 68CMA8
DT Conference; General Review
LA English
AB A review with .apprx.130 refs. The topics discussed include genomic organization, the **NS2-NS3** protease, and the NS3 protease.
RE.CNT 143 THERE ARE 143 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 32 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:230370 CAPLUS
DN 130:249231
TI Characterization of nonstructural protein features of the Flaviviridae: **HCV NS2-3 protease** activity and NS5A/5 phosphorylation
AU Soukhodolets, Karen Elaine
CS Washington Univ., St. Louis, MO, USA
SO (1998) 223 pp. Avail.: UMI, Order No. DA9905222
From: Diss. Abstr. Int., B 1999, 59(9), 4620
DT Dissertation
LA English
AB Unavailable

L8 ANSWER 34 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:514673 CAPLUS
DN 129:254236
TI Candidate targets for hepatitis C virus-specific antiviral therapy
AU Bartenschlager, Ralf
CS Institute for Virology, University of Mainz, Mainz, D-55131, Germany
SO Intervirology (1998), Volume Date 1997, 40(5-6), 378-393
CODEN: IVRYAK; ISSN: 0300-5526
PB S. Karger AG
DT Journal; General Review
LA English
AB A review with 122 refs. The **hepatitis C** virus (**HCV**) was identified as the major causative agent of posttransfusion and community-acquired **non-A, non-B hepatitis** throughout the world. It is an enveloped virus with a plus-strand RNA genome encoding a polyprotein of about 3,010 amino acids. This polyprotein is cleaved co- and

posttranslationally into mature viral proteins by host cell signal peptidases and 2 viral enzymes designated the **NS2-3 proteinase** and the NS3/4A **proteinase** complex. It is assumed that virus replication takes place in a membrane-associated complex containing at least 2 viral enzymic activities: the NS3 nucleoside triphosphatase (NTPase)/helicase and the NS5B RNA-dependent RNA polymerase (RdRp). Based on their important role for the viral life cycle and the wealth of information available for related cellular and viral proteins, the NS3/4A serine-type **proteinase** complex, the NS3 NTPase/helicase and the NS5B RdRp are the most attractive targets for development of **HCV**-specific antiviral therapies. This review will summarize our current knowledge about structure and function of these proteins and describe approaches pursued to identify effective antiviral compds.

RE.CNT 122 THERE ARE 122 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 35 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:247503 CAPLUS
DN 129:24718
TI Mechanism of autoproteolysis at the **NS2-NS3** junction of the hepatitis C virus polyprotein
AU Wu, Zhen; Yao, Nanhua; Le, Hung V.; Weber, Patricia C.
CS Schering-Plough Res. Inst., Kenilworth, NJ, 07033, USA
SO Trends in Biochemical Sciences (1998), 23(3), 92-94
CODEN: TBSCDB; ISSN: 0376-5067
PB Elsevier Science Ltd.
DT Journal
LA English
AB The authors report here the presence of zinc in NS3 implications for the processing of the HCV polyprotein, especially in the mechanism on **NS2-NS3** cleavage.

RE.CNT 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 39 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1994:264438 CAPLUS
DN 120:264438
TI A second **hepatitis C** virus-encoded **proteinase**
AU Grakoui, Arash; McCourt, David W.; Wychowski, Czeslaw; Feinstone, Stephen M.; Rice, Charles M.
CS Sch. Med., Washington Univ., St. Louis, MO, 63110-1093, USA
SO Proceedings of the National Academy of Sciences of the United States of America (1993), 90(22), 10583-7
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB Host and viral **proteinases** are believed to be required for the production of at least nine **hepatitis C** virus (**HCV**)-specific polyprotein cleavage products. Although several cleavages appear to be catalyzed by host signal peptidase or the **HCV** NS3 serine **proteinase**, the enzyme responsible for cleavage at the 2/3 site has not been identified. In this report, the authors have defined the 2/3 cleavage site and obtained evidence which suggests that this cleavage is mediated by a second **HCV**-encoded **proteinase**, located between aa 827 and 1207. This region encompasses the C-terminal portion of the 23-kDa **NS2** protein, the 2/3 cleavage site, and the serine **proteinase** domain of NS3. Efficient processing at the 2/3 site was observed in mammalian cells, Escherichia coli, and in plant or animal cell-free translation systems in

the absence of microsomal membranes. Cleavage at the 2/3 site was abolished by alanine substitutions for **NS2** residues His-952 or Cys-993 but was unaffected by several other substitution mutations, including those that inactivate NS3 serine **proteinase** function. Mutations abolishing cleavage at the 2/3 site did not block cleavage at other sites in the **HCV** polyprotein. Cotransfection expts. indicate that the 2/3 site can be cleaved in trans, which should facilitate purification and further characterization of this enzyme.

L8 ANSWER 41 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:485492 BIOSIS
DN PREV200000485492
TI Establishment of a cell-based assay for evaluation of compounds against **HCV NS2-3 protease** activity.
AU Wenzel, M. [Reprint author]; Troxell, J. [Reprint author]; Buckheit, R. W. [Reprint author]; Huang, M. [Reprint author]
CS Southern Res. Inst., Frederick, MD, USA
SO Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (1999) Vol. 39, pp. 409. cd-rom.
Meeting Info.: 39th Interscience Conference on Antimicrobial Agents and Chemotherapy. San Francisco, California, USA. September 26-29, 1999. American Society for Microbiology.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 8 Nov 2000
Last Updated on STN: 10 Jan 2002

L8 ANSWER 45 OF 45 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AN 96:606913 SCISEARCH
GA The Genuine Article (R) Number: VB800
TI PROCESSING PATHWAYS OF THE HEPATITIS-C VIRUS PROTEINS
AU LOHMANN V; KOCH J O; BARTENSCHLAGER R (Reprint)
CS UNIV MAINZ, INST VIROL, ZAHLBACHER STR 67, D-55131 MAINZ, GERMANY (Reprint); UNIV MAINZ, INST VIROL, D-55131 MAINZ, GERMANY
CYA GERMANY
SO JOURNAL OF HEPATOLOGY, (1996) Vol. 24, Supp. 2, pp. 11-19.
ISSN: 0169-5185.
DT Article; Journal
FS LIFE; CLIN
LA ENGLISH
REC Reference Count: 63
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB **Hepatitis C** virus (**HCV**) is the major etiological agent of posttransfusion and community-acquired **non-A, non-B hepatitis**, It is an enveloped virus, grouped as a separate genus in the Flaviviridae family, The plus-stranded RNA genome encodes a polyprotein of about 3000 amino acids with the structural proteins core, El and E2 residing in the amino terminal quarter of the polyprotein and the nonstructural proteins **NS2**, **NS3**, NS4A, NS4B, NS5A and NS5B in the remainder, Maturation of the structural proteins is mediated by host cell signalases located in the lumen of the endoplasmic reticulum and cleaving behind stretches of hydrophobic amino acids, At least two virally encoded **proteinases** are responsible for processing of the NS proteins: a zinc-dependent **metalloproteinase** encompassing the **NS2** domain and the amino terminal portion of NS3, which is essential for cleavage at the **NS2/3** junction; a serine-type **proteinase** located in the amino terminal domain of NS3 is required for cleavage at all sites downstream of the NS3 carboxy terminus, However,

although the NS3 domain contains proteolytic activity, with the exception of the NS5A/5B junction cleavage only occurs in the presence of NS4A. This 54 amino acid long peptide can modulate the proteolytic activity of the enzyme in cis and in tr ans, probably by the formation of a stable NS3/NS4A complex, Modulation of the **proteinase** activity may be a way to regulate the expression and replication of the **HCV** genome. (C) European Association for the Study of the Liver.

L8 ANSWER 4 OF 45 MEDLINE on STN
AN 2000044691 MEDLINE
DN PubMed ID: 10574908
TI Inhibition of hepatitis C virus **NS2/3** processing by NS4A peptides. Implications for control of viral processing.
AU Darke P L; Jacobs A R; Waxman L; Kuo L C
CS Department of Antiviral Research, Merck Research Laboratories, West Point, Pennsylvania 19486, USA.
SO Journal of biological chemistry, (1999 Dec 3) 274 (49) 34511-4. Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200002
ED Entered STN: 20000209
Last Updated on STN: 20000209
Entered Medline: 20000203
AB The **NS2/3 protease** of **hepatitis C** virus is responsible for a single cleavage in the viral polyprotein between the nonstructural proteins **NS2** and NS3. The minimal protein region necessary to catalyze this cleavage includes most of **NS2** and the N-terminal one-third of NS3. **Autocleavage** reactions using **NS2/3** protein translated in vitro are used here to investigate the inhibitory potential of peptides likely to affect the reaction. Peptides representing the cleaved sequence have no effect upon reaction rates, and the reaction rate is insensitive to dilution. Both results are consistent with prior suggestions that the **NS2/3** cleavage is an intramolecular reaction. Surprisingly, peptides containing the 12-amino acid region of NS4A responsible for binding to NS3 inhibit the **NS2/3** reaction with K(i) values as low as 3 microM. Unrelated peptide sequences of similar composition are not inhibitory, and neither are peptides containing incomplete segments of the NS4A region that binds to NS3. Inhibition of **NS2/3** by NS4A peptides can be rationalized from the organizing effect of NS4A on the N terminus of NS3 (the **NS2/3** cleavage point) as suggested by the known three-dimensional structure of the NS3 **protease** domain (Yan, Y., Li, Y., Munshi, S., Sardana, V., Cole, J. L., Sardana, M., Steinkuhler, C., Tomei, L., De Francesco, R., Kuo, L. C., and Chen, Z. (1998) Protein Sci. 7, 837-847). These findings may imply a sequential order to proteolytic maturation events in **hepatitis C** virus.

L8 ANSWER 5 OF 45 MEDLINE on STN
AN 2000039338 MEDLINE
DN PubMed ID: 10574181
TI The design and synthesis of potent inhibitors of **hepatitis C** virus NS3-4A **proteinase**.
AU Attwood M R; Bennett J M; Campbell A D; Canning G G; Carr M G; Conway E; Dunsdon R M; Greening J R; Jones P S; Kay P B; Handa B K; Hurst D N; Jennings N S; Jordan S; Keech E; O'Brien M A; Overton H A; King-Underwood J; Raynham T M; Stenson K P; Wilkinson C S; Wilkinson T C; Wilson F X
CS Department of Chemistry, Roche Discovery Welwyn, Welwyn Garden City, Hertfordshire, UK.
SO Antiviral chemistry & chemotherapy, (1999 Sep) 10 (5) 259-73. Journal code: 9009212. ISSN: 0956-3202.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals

EM 199912
ED Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991209
AB **Hepatitis C** virus (**HCV**) is the cause of the majority of transfusion-associated hepatitis and a significant proportion of community-acquired hepatitis worldwide. Infection by **HCV** frequently leads to persistent infections that result in a range of clinical conditions including an asymptomatic carrier state, severe chronic active hepatitis, cirrhosis and, in some cases, hepatocellular carcinoma. The **HCV** genome consists of a single-stranded, positive sense RNA containing an open reading frame of approximately 9060 nucleotides. This is translated into a single polyprotein of approximately 3020 amino acids (C-E1-E2-p7-**NS2-NS3**-NS4A-NS4B-NS5A-NS5B), which in turn is processed by a series of host and viral **proteinases** into at least 10 cleavage products. The N-terminal portion of the NS3 protein encodes a serine **proteinase** that is responsible for the cleavage at the NS3-4A, NS4A-4B, NS4B-5A and NS5A-5B junctions. The 54 amino acid NS4A protein is a cofactor that binds to the NS3 protein and enhances its proteolytic activity. This report describes the expression of a recombinant NS3-4A **proteinase** fusion protein in Escherichia coli and the in vitro characterization of the enzyme activity using synthetic peptide substrates. It then demonstrates how these results were employed to guide the design of potent inhibitors of this enzyme.

L8 ANSWER 7 OF 45 MEDLINE on STN
AN 1999143345 MEDLINE
DN PubMed ID: 9986797
TI Internal processing of hepatitis C virus NS3 protein.
AU Shoji I; Suzuki T; Sato M; Aizaki H; Chiba T; Matsuura Y; Miyamura T
CS Department of Virology II, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo, 162-8640, Japan.
SO Virology, (1999 Feb 15) 254 (2) 315-23.
Journal code: 0110674. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199903
ED Entered STN: 19990324
Last Updated on STN: 20000303
Entered Medline: 19990309
AB **Hepatitis C** virus (**HCV**) NS3 protein contains at least three enzymatic activities: **NS2-3 protease**, NS3 serine **protease**, and NTPase/RNA helicase. It has been shown that **NS2/3** cleavage is mediated by **NS2-3 protease**, whereas NS3 serine **protease** is responsible for the other four cleavage sites of the nonstructural (NS) region. In this study, we showed that the internal cleavage of NS3 protein produced two products of 49 kDa (NS3a) and 23 kDa (NS3b) when the entire NS3 region (aa 1027-1657) or the whole open reading frame (aa 1-3010) was expressed in mammalian and insect cells. By means of site-directed mutagenesis, we demonstrated that NS3a/NS3b cleavage occurs within the RNA helicase sequence motif that is highly conserved in the Flaviviridae family and that neither **NS2-3 protease** nor NS3 serine **protease** was responsible for this cleavage. The NS3 **protease** of flaviviruses, dengue virus type 2, for example, has been shown to mediate the internal cleavage of NS3. The NS3 proteins of **HCV** and dengue virus may thus be cleaved internally at the same sequence by different mechanisms of

proteolysis. Also discussed is a possible role for the internal processing of **HCV** NS3 in the viral life cycle and its pathogenesis.

L8 ANSWER 10 OF 45 MEDLINE on STN
AN 1998111692 MEDLINE
DN PubMed ID: 9450039
TI **Hepatitis C** virus **NS2-3 proteinase**.
AU Wilkinson C S
CS Roche Discovery Welwyn, Welwyn Garden City, Herts, England.
SO Biochemical Society transactions, (1997 Nov) 25 (4) S611.
Journal code: 7506897. ISSN: 0300-5127.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199804
ED Entered STN: 19980422
Last Updated on STN: 20000303
Entered Medline: 19980413

L8 ANSWER 12 OF 45 MEDLINE on STN
AN 97404642 MEDLINE
DN PubMed ID: 9261354
TI In vitro study of the **NS2-3 protease** of **hepatitis C** virus.
AU Pieroni L; Santolini E; Fipaldini C; Pacini L; Migliaccio G; La Monica N
CS I.R.B.M. Instituto di Ricerche di Biologia Molecolare P. Angeletti, Italy.
SO Journal of virology, (1997 Sep) 71 (9) 6373-80.
Journal code: 0113724. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199709
ED Entered STN: 19970926
Last Updated on STN: 20000303
Entered Medline: 19970917
AB Processing at the C terminus of the **NS2** protein of **hepatitis C** virus (**HCV**) is mediated by a virus-encoded **protease** which spans most of the **NS2** protein and part of the NS3 polypeptide. In vitro cotranslational cleavage at the 2-3 junction is stimulated by the presence of microsomal membranes and ultimately results in the membrane insertion of the **NS2** polypeptide. To characterize the biochemical properties of this viral **protease**, we have established an in vitro assay whereby the **NS2-3 protease** of **HCV** BK can be activated posttranslationally by the addition of detergents. The cleavage proficiency of several deletion and single point mutants was the same as that observed with microsomal membranes, indicating that the overall sequence requirements for proper cleavage at this site are maintained even under these artificial conditions. The processing efficiency of the **NS2-3 protease** varied according to the type of detergent used and its concentration. Also, the incubation temperature affected the cleavage at the 2-3 junction. The autoproteolytic activity of the **NS2-3 protease** could be inhibited by alkylating agents such as iodoacetamide and

N-ethylmaleimide. Metal chelators such as EDTA and phenanthroline also inhibited the viral enzyme. The EDTA inhibition of **NS2-3** cleavage could be reversed, at least in part, by the addition of ZnCl2 and CdCl2. Among the common **protease** inhibitors tested, tosyl phenylalanyl chloromethyl ketone and soybean trypsin inhibitor inactivated the **NS2-3 protease**. By means of gel filtration analysis, it was observed that the redox state of the reaction mixture greatly influenced the processing efficiency at the 2-3 site and that factors present in the rabbit reticulocyte lysate, wheat germ extract, and HeLa cell extract were required for efficient processing at this site. Thus, the in vitro assay should allow further characterization of the biochemical properties of the **NS2-3 protease** of **HCV** and the identification of host components that contribute to the efficient processing at the 2-3 junction.

L8 ANSWER 17 OF 45 MEDLINE on STN
AN 96266415 MEDLINE
DN PubMed ID: 8661414
TI In vitro studies on the activation of the **hepatitis C** virus NS3 **proteinase** by the NS4A cofactor.
AU Koch J O; Lohmann V; Herian U; Bartenschlager R
CS Institute for Virology, Johannes-Gutenberg University Mainz, Germany.
SO Virology, (1996 Jul 1) 221 (1) 54-66.
Journal code: 0110674. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199608
ED Entered STN: 19960822
Last Updated on STN: 19960822
Entered Medline: 19960814
AB Proteolytic processing of the nonstructural proteins of the **hepatitis C** virus (**HCV**) is mediated by two viral **proteinases**: the **NS2-3 proteinase** cleaving at the **NS2/3** junction and the NS3 serine-type **proteinase** responsible for processing at the NS3/4A, NS4A/B, NS4B/5A, and NS5A/B sites. Activity of the NS3 **proteinase** is modulated by NS4A. In the absence of this cofactor processing at the NS3-dependent sites does not occur or, in the case of the NS5A/B junction, is poor but increased when NS4A is present. Although recent studies demonstrated that **proteinase** activation requires direct interaction between NS3 and NS4A, the mechanism by which NS4A exerts the activation function is not known. To further analyze the conditions of **proteinase** activation and to characterize the NS3 sequences important for complex formation and activation we used an in vitro assay in which radiolabeled **HCV** substrates were mixed with NS3 **proteinase** and synthetic NS4A peptides. We found that microsomal membranes are not required for **proteinase** activation. However, they are important for efficient accessibility of the NS4A/B site but not the other trans-cleavage sites. Studies with NS3 deletion mutants identified a region between amino acids 15 and 22 which is essential for **proteinase** activation. Results obtained with several mutations introduced into this sequence show that a weak overall association between NS3 and NS4A is sufficient for **proteinase** activation and suggest that a beta-sheet at the NS3 amino terminus plays an important role. Although not essential for **proteinase** activation the amino terminal 14 NS3 residues were found to have an auxiliary function probably by stabilizing the NS3/4A interaction. Finally, we could demonstrate

intracellular, peptide-mediated modulation of **proteinase** activity providing the basis for the development of a novel therapeutic concept.

L8 ANSWER 21 OF 45 MEDLINE on STN
AN 95088591 MEDLINE
DN PubMed ID: 7996139
TI Analysis of NS3-mediated processing of the hepatitis C virus non-structural region in vitro.
AU D'Souza E D; O'Sullivan E; Amphlett E M; Rowlands D J; Sangar D V; Clarke B E
CS Department of Molecular Sciences, Wellcome Research Laboratories, Beckenham, Kent, U.K.
SO Journal of general virology, (1994 Dec) 75 (Pt 12) 3469-76.
Journal code: 0077340. ISSN: 0022-1317.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199501
ED Entered STN: 19950126
Last Updated on STN: 20000303
Entered Medline: 19950113
AB The **protease** activity of the **hepatitis C** virus (**HCV**) NS3 protein has been investigated using transient expression methods in mammalian cells, as well as in vitro transcription/translation systems. We confirmed that expression of the NS3-5 polyprotein in rabbit reticulocyte lysates results in efficient cis processing at the NS3/NS4 junction. However, processing at the other predicted sites of NS3-mediated cleavage varied markedly in efficiency, the site most susceptible being that between NS5A and NS5B. Time-course analysis of the proteolytic processing of the **HCV** non-structural precursor showed that the cis cleavage between NS3 and NS4 occurred extremely rapidly. However, efficient cleavage at this position was dependent on the prior removal of the **NS2** protein. Furthermore, the presence of uncleaved **NS2** sequences on the enzyme severely impeded NS3-mediated proteolysis at downstream sites in the polyprotein. This suggests therefore that efficient cleavage at the **NS2/NS3** junction is a pivotal event in **HCV** replication. During the course of this study a proteolytically inactive mutant of NS3 was characterized carrying a previously unreported amino acid substitution near the proposed active site of the enzyme. Molecular modelling suggested that the amino acid present at this position may influence the conformation of the active site of the enzyme. Recently a number of reports have described a second **protease** activity, located in the **NS2/NS3** region, which is responsible for cleavage at the **NS2/NS3** junction. We have identified an isolate of **HCV**, obtained from a U.K. patient, which has a virtually inactive **NS2/NS3 protease**. The possible implications of this observation are discussed.

L8 ANSWER 28 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:90406 CAPLUS
DN 132:290272
TI Expression and characterization of the **HCV NS2 protease**
AU Reed, Karen E.; Rice, Charles M.
CS Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA
SO Methods in Molecular Medicine (1998), 19(Hepatitis C Protocols), 331-342

CODEN: MMMEFN
PB Humana Press Inc.
DT Journal
LA English
AB This article describes the use of cell-free transcription and translation (rabbit reticulocyte lysate) systems for the expression and characterization of the **hepatitis C** virus **NS2 protease**.
RE.CNT 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 29 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:604367 CAPLUS
DN 132:46612
TI **Proteases** of the **hepatitis C** virus
AU Urbani, Andrea; De Francesco, Raffaele; Steinkuhler, Christian
CS Inst. di Ricerche di Biologia Molecolare (IRBM) P. Angeletti, Rome, Italy
SO Proteases of Infectious Agents (1999), 61-91. Editor(s): Dunn, Ben M. Publisher: Academic, San Diego, Calif.
CODEN: 68CMA8
DT Conference; General Review
LA English
AB A review with .apprx.130 refs. The topics discussed include genomic organization, the **NS2-NS3** protease, and the NS3 protease.
RE.CNT 143 THERE ARE 143 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 32 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:230370 CAPLUS
DN 130:249231
TI Characterization of nonstructural protein features of the Flaviviridae: **HCV NS2-3 protease** activity and NS5A/5 phosphorylation
AU Soukhodolets, Karen Elaine
CS Washington Univ., St. Louis, MO, USA
SO (1998) 223 pp. Avail.: UMI, Order No. DA9905222
From: Diss. Abstr. Int., B 1999, 59(9), 4620
DT Dissertation
LA English
AB Unavailable

L8 ANSWER 34 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:514673 CAPLUS
DN 129:254236
TI Candidate targets for hepatitis C virus-specific antiviral therapy
AU Bartenschlager, Ralf
CS Institute for Virology, University of Mainz, Mainz, D-55131, Germany
SO Intervirology (1998), Volume Date 1997, 40(5-6), 378-393
CODEN: IVRYAK; ISSN: 0300-5526
PB S. Karger AG
DT Journal; General Review
LA English
AB A review with 122 refs. The **hepatitis C** virus (**HCV**) was identified as the major causative agent of posttransfusion and community-acquired **non-A**, **non-B hepatitis** throughout the world. It is an enveloped virus with a plus-strand RNA genome encoding a polyprotein of about 3,010 amino acids. This polyprotein is cleaved co- and

posttranslationally into mature viral proteins by host cell signal peptidases and 2 viral enzymes designated the **NS2-3 proteinase** and the NS3/4A **proteinase** complex. It is assumed that virus replication takes place in a membrane-associated complex containing at least 2 viral enzymic activities: the NS3 nucleoside triphosphatase (NTPase)/helicase and the NS5B RNA-dependent RNA polymerase (RdRp). Based on their important role for the viral life cycle and the wealth of information available for related cellular and viral proteins, the NS3/4A serine-type **proteinase** complex, the NS3 NTPase/helicase and the NS5B RdRp are the most attractive targets for development of **HCV**-specific antiviral therapies. This review will summarize our current knowledge about structure and function of these proteins and describe approaches pursued to identify effective antiviral compds.

RE.CNT 122 THERE ARE 122 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 35 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:247503 CAPLUS
DN 129:24718
TI Mechanism of autoproteolysis at the **NS2-NS3** junction of the hepatitis C virus polyprotein
AU Wu, Zhen; Yao, Nanhua; Le, Hung V.; Weber, Patricia C.
CS Schering-Plough Res. Inst., Kenilworth, NJ, 07033, USA
SO Trends in Biochemical Sciences (1998), 23(3), 92-94
CODEN: TBSCDB; ISSN: 0376-5067
PB Elsevier Science Ltd.
DT Journal
LA English
AB The authors report here the presence of zinc in NS3 implications for the processing of the HCV polyprotein, especially in the mechanism on **NS2-NS3** cleavage.

RE.CNT 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 39 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1994:264438 CAPLUS
DN 120:264438
TI A second **hepatitis C** virus-encoded **proteinase**
AU Grakoui, Arash; McCourt, David W.; Wychowski, Czeslaw; Feinstone, Stephen M.; Rice, Charles M.
CS Sch. Med., Washington Univ., St. Louis, MO, 63110-1093, USA
SO Proceedings of the National Academy of Sciences of the United States of America (1993), 90(22), 10583-7
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB Host and viral **proteinases** are believed to be required for the production of at least nine **hepatitis C** virus (**HCV**)-specific polyprotein cleavage products. Although several cleavages appear to be catalyzed by host signal peptidase or the **HCV** NS3 serine **proteinase**, the enzyme responsible for cleavage at the 2/3 site has not been identified. In this report, the authors have defined the 2/3 cleavage site and obtained evidence which suggests that this cleavage is mediated by a second **HCV**-encoded **proteinase**, located between aa 827 and 1207. This region encompasses the C-terminal portion of the 23-kDa **NS2** protein, the 2/3 cleavage site, and the serine **proteinase** domain of NS3. Efficient processing at the 2/3 site was observed in mammalian cells, Escherichia coli, and in plant or animal cell-free translation systems in

the absence of microsomal membranes. Cleavage at the 2/3 site was abolished by alanine substitutions for **NS2** residues His-952 or Cys-993 but was unaffected by several other substitution mutations, including those that inactivate NS3 serine **proteinase** function. Mutations abolishing cleavage at the 2/3 site did not block cleavage at other sites in the **HCV** polyprotein. Cotransfection expts. indicate that the 2/3 site can be cleaved in trans, which should facilitate purification and further characterization of this enzyme.

L8 ANSWER 41 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:485492 BIOSIS
DN PREV200000485492
TI Establishment of a cell-based assay for evaluation of compounds against **HCV NS2-3 protease** activity.
AU Wenzel, M. [Reprint author]; Troxell, J. [Reprint author]; Buckheit, R. W. [Reprint author]; Huang, M. [Reprint author]
CS Southern Res. Inst., Frederick, MD, USA
SO Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (1999) Vol. 39, pp. 409. cd-rom.
Meeting Info.: 39th Interscience Conference on Antimicrobial Agents and Chemotherapy. San Francisco, California, USA. September 26-29, 1999. American Society for Microbiology.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 8 Nov 2000
Last Updated on STN: 10 Jan 2002

L8 ANSWER 45 OF 45 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AN 96:606913 SCISEARCH
GA The Genuine Article (R) Number: VB800
TI PROCESSING PATHWAYS OF THE HEPATITIS-C VIRUS PROTEINS
AU LOHMANN V; KOCH J O; BARTENSCHLAGER R (Reprint)
CS UNIV MAINZ, INST VIROL, ZAHLBACHER STR 67, D-55131 MAINZ, GERMANY (Reprint); UNIV MAINZ, INST VIROL, D-55131 MAINZ, GERMANY
CYA GERMANY
SO JOURNAL OF HEPATOLOGY, (1996) Vol. 24, Supp. 2, pp. 11-19.
ISSN: 0169-5185.
DT Article; Journal
FS LIFE; CLIN
LA ENGLISH
REC Reference Count: 63
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB **Hepatitis C** virus (**HCV**) is the major etiological agent of posttransfusion and community-acquired **non-A, non-B hepatitis**, It is an enveloped virus, grouped as a separate genus in the Flaviviridae family, The plus-stranded RNA genome encodes a polyprotein of about 3000 amino acids with the structural proteins core, El and E2 residing in the amino terminal quarter of the polyprotein and the nonstructural proteins **NS2**, **NS3**, NS4A, NS4B, NS5A and NS5B in the remainder, Maturation of the structural proteins is mediated by host cell signalases located in the lumen of the endoplasmic reticulum and cleaving behind stretches of hydrophobic amino acids, At least two virally encoded **proteinases** are responsible for processing of the NS proteins: a zinc-dependent **metalloproteinase** encompassing the **NS2** domain and the amino terminal portion of NS3, which is essential for cleavage at the **NS2/3** junction; a serine-type **proteinase** located in the amino terminal domain of NS3 is required for cleavage at all sites downstream of the NS3 carboxy terminus, However,

although the NS3 domain contains proteolytic activity, with the exception of the NS5A/5B junction cleavage only occurs in the presence of NS4A. This 54 amino acid long peptide can modulate the proteolytic activity of the enzyme in cis and in tr ans, probably by the formation of a stable NS3/NS4A complex, Modulation of the **proteinase** activity may be a way to regulate the expression and replication of the **HCV** genome. (C) European Association for the Study of the Liver.

L11 ANSWER 6 OF 18 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2002:466208 CAPLUS
DN 137:29823
TI Purification of active NS2/3 **protease** of **hepatitis C** virus from inclusion bodies
IN Thibeault, Diane; Lamarre, Daniel; Maurice, Roger; Pilote, Louise; Pause, Arnim
PA Boehringer Ingelheim (Canada) Ltd., Can.
SO PCT Int. Appl., 67 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002048375	A2	20020620	WO 2001-CA1796	20011213
	WO 2002048375	A3	20030227		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2002024688	A5	20020624	AU 2002-24688	20011213
	EP 1343897	A2	20030917	EP 2001-994573	20011213
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	JP 2004514459	T2	20040520	JP 2002-550090	20011213
	US 2002192640	A1	20021219	US 2001-17736	20011214
	US 2004077066	A1	20040422	US 2003-650585	20030828
PRAI	US 2000-256031P	P	20001215		
	WO 2001-CA1796	W	20011213		
	US 2001-17736	A3	20011214		

AB A method for producing a refolded, inactive form of recombinantly produced NS2/3 **protease** by purifying the **protease** from inclusion bodies in the presence of a chaotropic agent and refolding the purified **protease** by contacting it with a reducing agent and lauryldiethylamine oxide (LDAO) in the presence of reduced concentration of chaotropic agent or polar additive. The invention further comprises a method for activating this refolded inactive NS2/3 **protease** by adding an activation detergent. This method produces large amts. of the active NS2/3 **protease** to allow small mols. and ligands to be screened as potential inhibitors of NS2/3 **protease**, which may be useful as therapeutic agents against **HCV**. Protocols for the manufacture and resolubilization of the enzyme as inclusion bodies in Escherichia coli are described in detail.

L11 ANSWER 8 OF 18 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN DUPLICATE 2
AN 2003451824 EMBASE
TI In Vitro Characterization of a Purified NS2/3 **Protease** Variant of **Hepatitis C** Virus.
AU Thibeault D.; Maurice R.; Pilote L.; Lamarre D.; Pause A.
CS D. Thibeault, Dept. of Biological Sciences, Boehringer Ingelheim (Canada) Ltd., Research and Development, 2100 Cunard St., Laval, Que. H7S 2G5, Canada. dthibeault@lav.boehringer-ingelheim.com
SO Journal of Biological Chemistry, (7 Dec 2001) 276/49 (46678-46684). Refs: 36
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The cleavage of the **hepatitis C** virus polyprotein between the nonstructural proteins NS2 and NS3 is mediated by the NS2/3 **protease**, whereas the NS3 **protease** is responsible for the cleavage of the downstream proteins. Purification and in vitro characterization of the NS2/3 **protease** has been hampered by its hydrophobic nature. NS2/3 **protease** activity could only be detected in cells or in in vitro translation assays with the addition of microsomal membranes or detergent. To facilitate purification of this poorly characterized **protease**, we truncated the N-terminal hydrophobic domain, resulting in an active enzyme with improved biophysical properties. We define a minimal catalytic region of NS2/3 **protease** retaining **autocleavage** activity that spans residues 904-1206 and includes the C-terminal half of NS2 and the N-terminal NS3 **protease** domain. The NS2/3 (904-1206) variant was purified from Escherichia coli inclusion bodies and refolded by gel filtration chromatography. The purified inactive form of NS2/3 (904-1206) was activated by the addition of glycerol and detergent to induce **autocleavage** at the predicted site between Leu(1026) and Ala(1027). NS2/3 (904-1206) activity was dependent on zinc ions and could be **inhibited** by NS4A peptides, peptides that span the cleavage site, or an N-terminal peptidic cleavage product. This NS2/3 variant will facilitate the development of an assay suitable for identifying **inhibitors** of **HCV** replication.

L11 ANSWER 13 OF 18 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:94623 CAPLUS
DN 132:146019
TI Protease inhibitors: Current status and future prospects
AU Leung, Donmienne; Abbenante, Giovanni; Fairlie, David P.
CS Centre for Drug Design and Development, University of Queensland, Brisbane, 4072, Australia
SO Journal of Medicinal Chemistry (2000), 43(3), 305-341
CODEN: JMCMAR; ISSN: 0022-2623
PB American Chemical Society
DT Journal; General Review
LA English
AB A review with 354 refs. is given on Asp **protease inhibitors** (HIV-1 **protease**, renin, plasmepsins, cathepsin D, secreted Asp **protease**), Ser **protease inhibitors** (thrombin, factor Xa, elastase, tryptase, complement convertases, **hepatitis C**-NS3 **protease**, broad-spectrum Ser **protease inhibitors**), Cys **protease inhibitors** (cathepsin K, B, and L, caspases,

rhinovirus 3C **protease**, calpains), and **metalloprotease inhibitors** (angiotensin-converting enzyme, neutral endopeptidase, matrix **metalloprotease inhibitors**, tumor necrosis factor-α-converting enzyme).
RE.CNT 358 THERE ARE 358 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 15 OF 18 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3
AN 1999:798787 CAPLUS
DN 132:105173
TI Inhibition of hepatitis C virus NS2/3 processing by NS4A peptides. Implications for control of viral processing
AU Darke, Paul L.; Jacobs, Amanda R.; Waxman, Lloyd; Kuo, Lawrence C.
CS Department of Antiviral Research, Merck Research Laboratories, West Point, PA, 19486, USA
SO Journal of Biological Chemistry (1999), 274(49), 34511-34514
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB The NS2/3 **protease** of **hepatitis C** virus is responsible for a single cleavage in the viral polyprotein between the nonstructural proteins NS2 and NS3. The minimal protein region necessary to catalyze this cleavage includes most of NS2 and the N-terminal one-third of NS3. **Autocleavage** reactions using NS2/3 protein translated in vitro are used here to investigate the **inhibitory** potential of peptides likely to affect the reaction. Peptides representing the cleaved sequence have no effect upon reaction rates, and the reaction rate is insensitive to dilution Both results are consistent with prior suggestions that the NS2/3 cleavage is an intramol. reaction. Surprisingly, peptides containing the 12-amino acid region of NS4A responsible for binding to NS3 inhibit the NS2/3 reaction with Ki values as low as 3 μM. Unrelated peptide sequences of similar composition are not inhibitory, and neither are peptides containing incomplete segments of the NS4A region that binds to NS3. Inhibition of NS2/3 by NS4A peptides can be rationalized from the organizing effect of NS4A on the N terminus of NS3 (the NS2/3 cleavage point) as suggested by the known 3-dimensional structure of the NS3 **protease** domain. These findings may imply a sequential order to proteolytic maturation events in **hepatitis C** virus.
RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 18 OF 18 MEDLINE on STN DUPLICATE 4
AN 93323208 MEDLINE
DN PubMed ID: 8392606
TI Two distinct **proteinase** activities required for the processing of a putative nonstructural precursor protein of **hepatitis C** virus.
AU Hijikata M; Mizushima H; Akagi T; Mori S; Kakiuchi N; Kato N; Tanaka T; Kimura K; Shimotohno K
CS Virology Division, National Cancer Center Research Institute, Tokyo, Japan.
SO Journal of virology, (1993 Aug) 67 (8) 4665-75.
Journal code: 0113724. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-D11397

EM 199308
ED Entered STN: 19930826
Last Updated on STN: 20000303
Entered Medline: 19930816

AB Gene products of **hepatitis C** virus (**HCV**), a possible major causative agent of posttransfusion **non-A**, **non-B hepatitis**, are considered to be produced from a precursor polyprotein via proteolytic processing mediated by either host cell or viral **proteinases**. The presence of **HCV** serine **proteinase** has been proposed from analyses of amino acid sequence homology. To examine the processing mechanism of the **HCV** precursor polyprotein, the amino-terminal region of the putative nonstructural protein region of the **HCV** genome, containing the serine **proteinase** motif, was expressed and analyzed by using an in vitro transcription/translation system and a transient expression system in cultured cells. Two distinct **proteinase** activities which function in the production of a 70-kDa protein (p70) from the precursor polyprotein were detected. One of these **proteinase** activities, which cleaved the carboxyl (C)-terminal side of p70, required the presence of the serine **proteinase** motif, which is located in the amino (N)-terminal region of p70. That suggested that the predicted **HCV** serine **proteinase** was functional. The other activity, which was responsible for the cleavage of the N-terminal side of p70, required the expression of the region upstream and downstream of that cleavage site, including the p70 serine **proteinase** domain. From the results of pulse-chase analysis, using **proteinase inhibitors** coupled with a point mutation analysis, the latter activity was proposed to be a novel zinc-dependent **metalloproteinase**.